# Using VIP*T*-Jump to Distinguish Between Different Folding Mechanisms: Application to BBL and a Trpzip

Chun–Wei Lin,[†] Robert M. Culik,[‡] and Feng Gai[†,*]

[†]Department of Chemistry and [‡]Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104

**Table S1.** Name and sequence of the peptides studied.

| Name | Sequence |
|---|---|
| BBL | ALSPAIRRLLAEHNLDASAIKGTGVGGRLTREDVEKHLAK-$NH_2$ |
| Trpzip-2c | AWAWENGKWAWK-$NH_2$ |

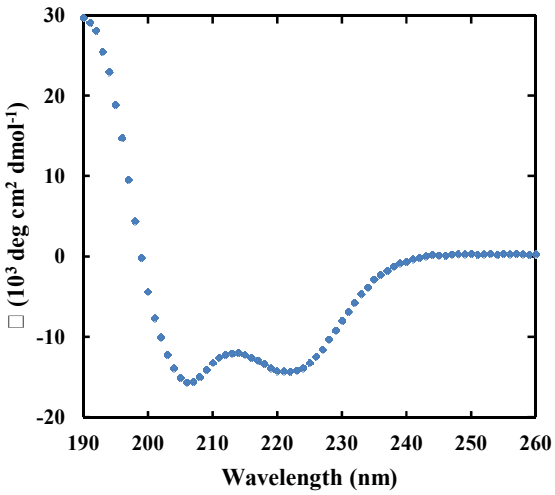


**Figure S1.** Far-UV CD spectrum of BBL, collected at 1°C.

**Table S2.** The equilibrium population percentage of each state (F: folded state, I: intermediate state, and U: unfolded state) corresponding to the free energies in Figure 2.

| | Temperature (K) | [F]% | [I]% | [U]% |
|---|---|---|---|---|
| **A** | 298.0 | 80.55 | 17.97 | 1.48 |
| | 310.5 | 70.33 | 20.15 | 9.52 |
| | 323.0 | 42.24 | 15.54 | 42.23 |
| **B** | 298.0 | 80.55 | 17.97 | 1.48 |
| | 310.5 | 70.33 | 20.15 | 9.52 |
| | 323.0 | 42.24 | 15.54 | 42.23 |

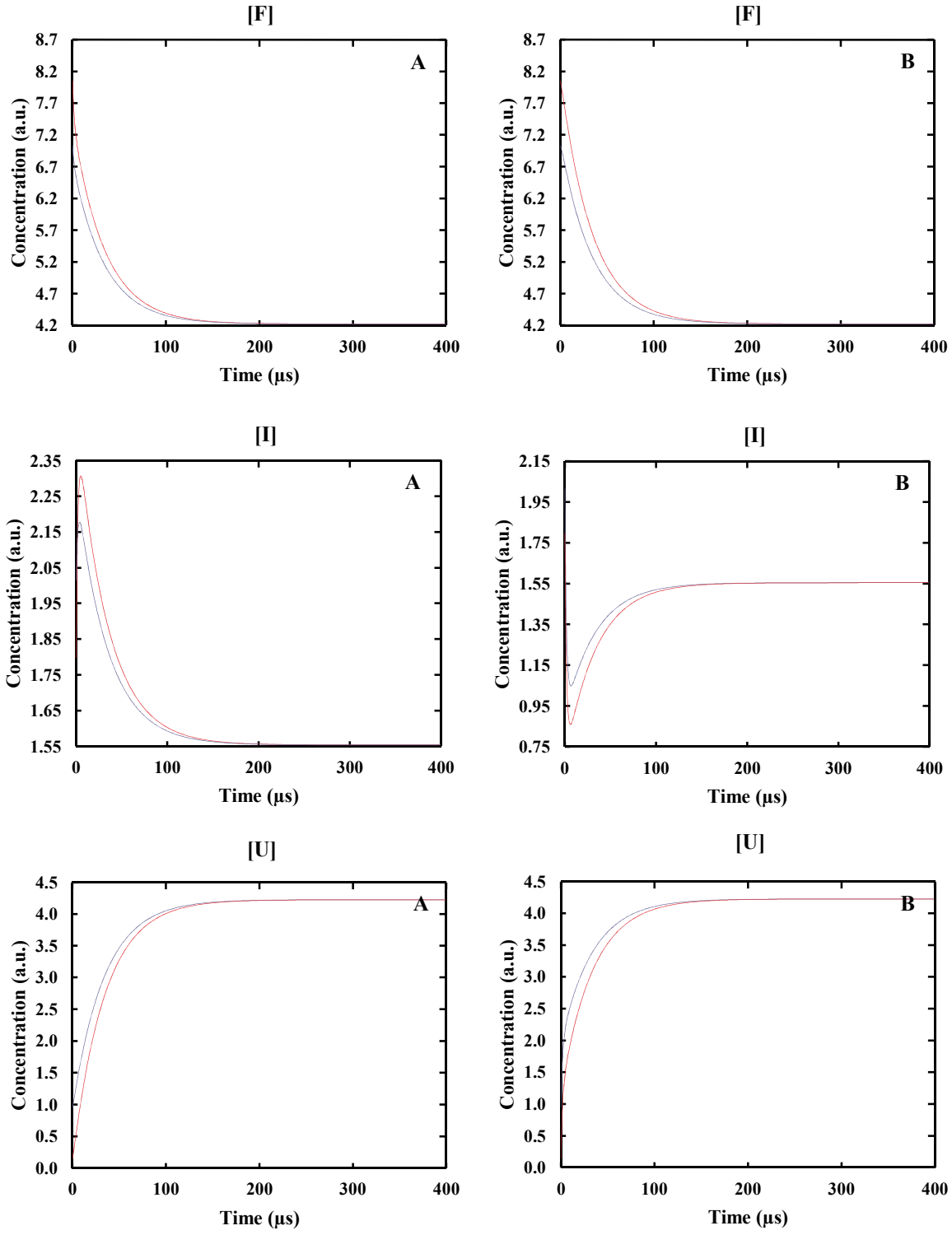


**Figure S2.** Relative population changes in response to a *T*-jump of 298 to 323 K (red) or 310.5 to 323 K (blue), for the two three-state folding mechanisms (A or B, as indicated) presented in Figure 2.

**Table S3.** *T*-jump relaxation kinetic parameters calculated for the two three-state folding seniors presented in Figure 2.

| Mechanism | *T*-jump | | Relaxation parameters of the folded state | | | |
|---|---|---|---|---|---|---|
| | $T_i$ (K) | $T_f$ (K) | $\tau_1$(μs) | $\tau_2$(μs) | $A_1$% | $A_2$% |
| A | 310.5 | 323.0 | 1.85 | 33.96 | 8.87 | 91.13 |
| | 298.0 | 323.0 | 1.85 | 33.96 | 16.10 | 83.90 |
| B | 310.5 | 323.0 | 34.28 | - | - | - |
| | 298.0 | 323.0 | 34.28 | - | - | - |
| Mechanism | *T*-jump | | Relaxation parameters of the intermediate state | | | |
| | $T_i$ (K) | $T_f$ (K) | $\tau_1$(μs) | $\tau_2$(μs) | $A_1$% | $A_2$% |
| A | 310.5 | 323.0 | 1.85 | 33.96 | 27.21 | 72.79 |
| | 298.0 | 323.0 | 1.85 | 33.96 | 42.42 | 57.58 |
| B | 310.5 | 323.0 | 1.85 | 33.96 | 63.07 | 36.93 |
| | 298.0 | 323.0 | 1.85 | 33.96 | 56.08 | 43.92 |
| Mechanism | *T*-jump | | Relaxation parameters of the unfolded state | | | |
| | $T_i$ (K) | $T_f$ (K) | $\tau_1$(μs) | $\tau_2$(μs) | $A_1$% | $A_2$% |
| A | 310.5 | 323.0 | 34.03 | - | - | - |
| | 298.0 | 323.0 | 34.03 | - | - | - |
| B | 310.5 | 323.0 | 1.846 | 33.96 | 30.78 | 69.22 |
| | 298.0 | 323.0 | 1.846 | 33.96 | 24.95 | 75.05 |

**Determination of the temperature dependence of the potential energy surface of BBL**

To determine the temperature dependence of the folding free energy surface of BBL, we first fit the surface obtained at 298 K by Wang and coworkers[1] to a 10th order polynomial function, which yielded the following equation:

$$G(q,T) = 0.18402 \cdot z^{10} - 0.16043 \cdot z^{9} - 1.0419 \cdot z^{8} + 0.63745 \cdot z^{7}$$
$$+2.1744 \cdot z^{6} - 0.40374 \cdot z^{5} - 2.1289 \cdot z^{4} - 0.91441 \cdot z^{3} + 1.701 \cdot z^{2}$$
$$+4.2706 \cdot z + 5.4721$$

where $z$ = (*rmsd* − 10.565)/5.824. To estimate the temperature dependence, we added a linear term, $m(T)$*(*rmsd* − 2.05577), to the above equation. To determine $m(T)$,we assumed, based on the CD melting curve (Figure S3), that at 287 K and 353 K the values of the population-averaged nativeness, <*Q*>, are 0.9 and 0.1, respectively, and then converted these values to population-averaged *rmsd*, <*rmsd*>, as was done previously by Wang and coworkers.[1] A third <*rmsd*> value, at 298 K, was further obtained from the free energy surface of Wang and coworkers. Finally, fitting these three <*rmsd*> points to a quadratic function allows us to determine $m(T)$. While we chose *rmsd* as the reaction coordinate for *G* in this manuscript, we also tried additional coordinates, including *Q* and $R_g$, for completeness. Furthermore, we created an arbitrary free energy landscape, analogous to the work in Xu, Purkayastha, Gai *J. Am. Chem. Soc.* **2006**, and ran simulations using this. We found no significant differences in the observed trends from our LD simulations for any of these landscapes. We also show

that the CD melting curve matches well to the IR melting curve obtained from following the absorbance of BBL at 1668 $cm^{-1}$ as a function of temperature (Figure S3).

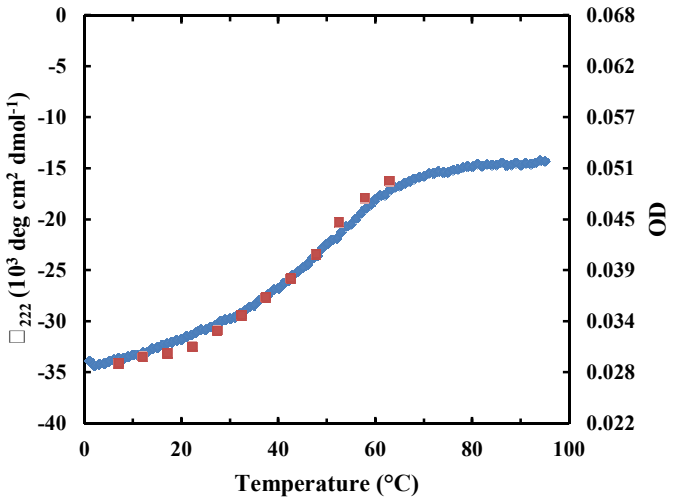


**Figure S3.** Overlay of CD thermal melting curve of BBL (blue) with IR melting curve probed at 1668 $cm^{-1}$ (red).

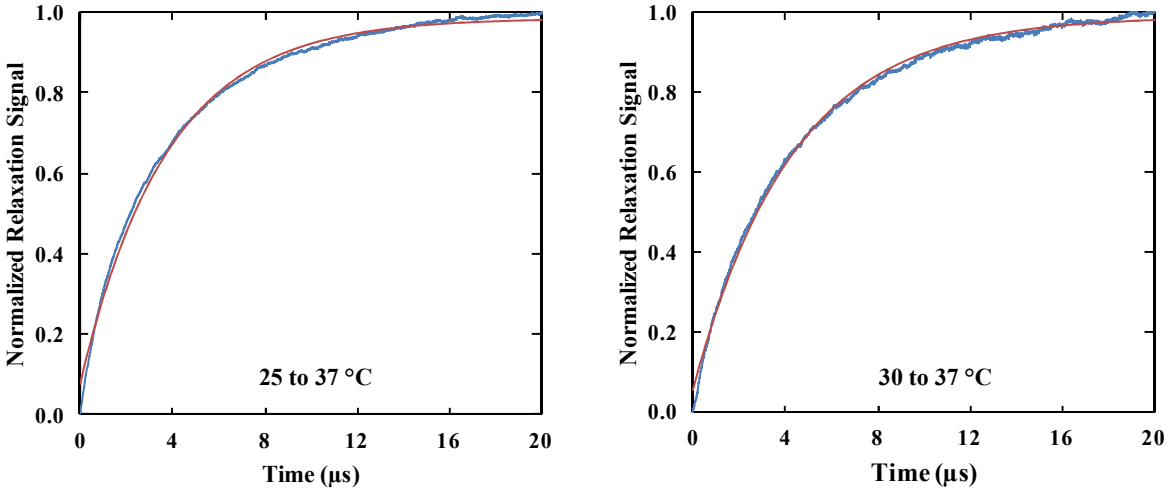


**Figure S4.** Single exponential fits of the Langevin Dynamics signal traces in Figure 3, with temperature jumps as indicated.

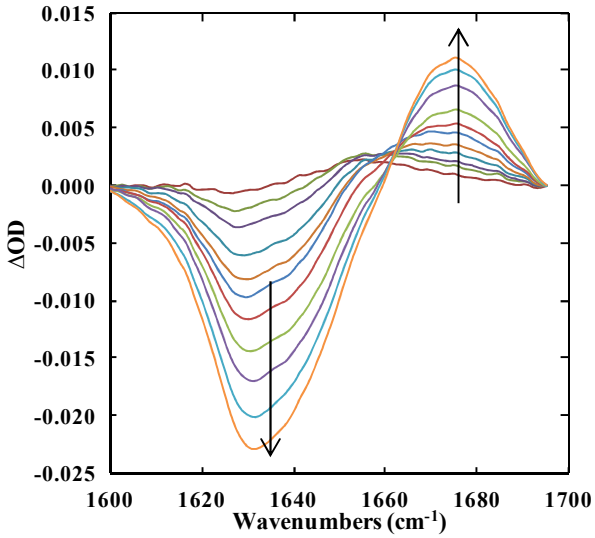


**Figure S5.** Difference FTIR spectra of BBL in the amide I' region, generated by subtracting the FTIR spectrum at 6.9 °C from those measured at higher temperatures (the highest temperature was 62.9 °C).

**Table S4.** Relaxation times of BBL obtained from fitting the corresponding *T*-jump IR relaxation kinetics to a single-exponential function.

| Probe Frequency ($cm^{-1}$) | $T_f$ | $T_i$ | $T_f$ - $T_i$ | $\tau_R$ (μs) | Rel. Amp. |
|---|---|---|---|---|---|
| 1668 | 46.8 | 38.1 | 8.7 | 3.3 ± 0.24 | 1.45 |
| | | 41.1 | 5.7 | 4.5 ± 0.04 | 1.00 |
| | 50.7 | 44.4 | 6.3 | 2.6 ± 0.05 | 3.39 |
| | | 46.5 | 4.2 | 3.0 ± 0.13 | 2.22 |
| | | 49.2 | 1.5 | 3.4 ± 0.02 | 1.00 |
| | 57.0 | 44.4 | 12.6 | 2.0 ± 0.18 | 2.05 |
| | | 53.3 | 3.7 | 2.8 ± 0.01 | 1.00 |
| 1630 | 46.5 | 39.2 | 7.3 | 3.4 ± 0.27 | 2.16 |
| | | 43.6 | 2.9 | 4.9 ± 0.13 | 1.00 |

**Table S5.** Relaxation times of Trpzip-2c obtained from fitting the corresponding *T*-jump IR relaxation kinetics to a single-exponential function.

| Probing Frequency ($cm^{-1}$) | $T_f$ | $T_i$ | $T_f - T_i$ | $\tau_R$ (μs) | Rel. Amp. |
|---|---|---|---|---|---|
| 1630 | 61.8 | 46.8 | 15.0 | 0.90 ± 0.03 | 1.56 |
| | | 52.9 | 9.0 | 0.92 ± 0.06 | 1.00 |
| | 72.4 | 52.5 | 19.9 | 0.50 ± 0.10 | - |
| | 57.8 | 41.1 | 16.7 | 1.10 ± 0.14 | - |

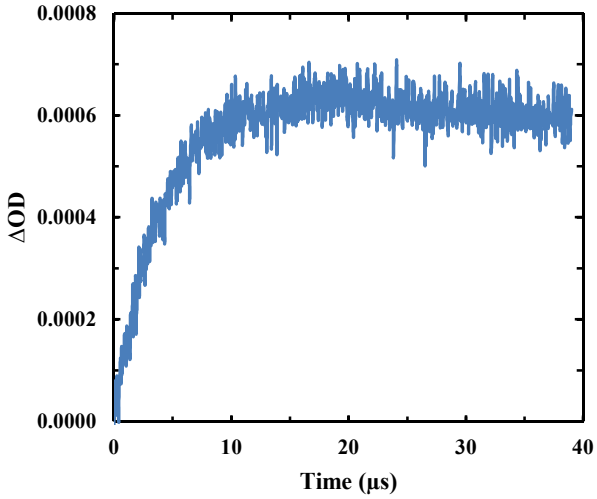


**Figure S6.** Conformational relaxation kinetics of BBL obtained with a probing frequency of 1668 $cm^{-1}$ after a *T*-jump from 49.2 to 50.7 °C.

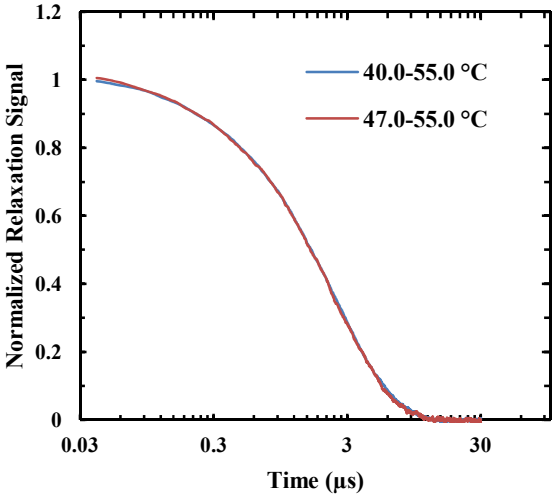


**Figure S7.** Simulated relaxation kinetics in response to *T*-jumps from different initial temperatures to the same final temperature, as indicated, for an incipient downhill folding scenario (Figure S8).

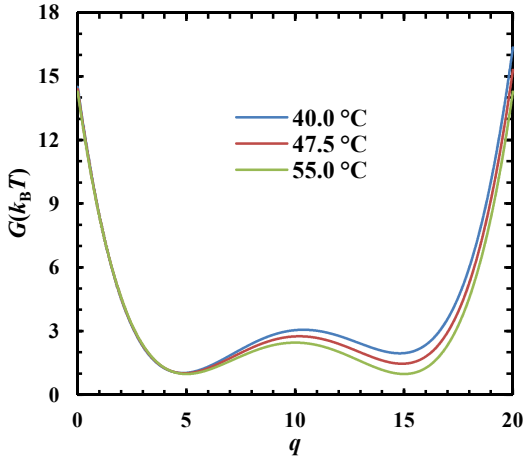


**Figure S8.** Free energy surfaces for an incipient downhill folding scenario used to simulate the relaxation kinetics in Figure S7.

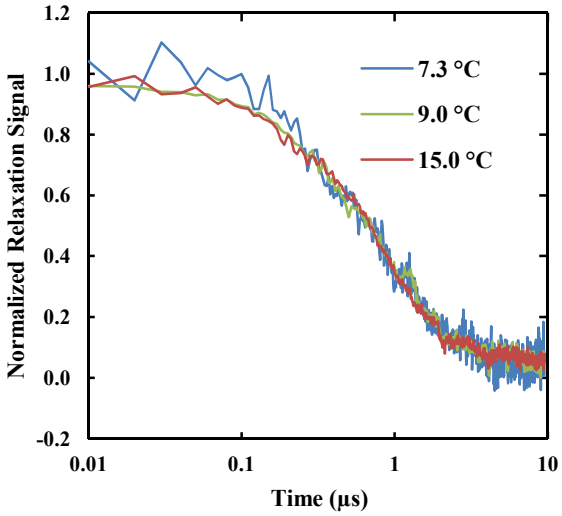


**Figure S9.** Relaxation kinetics of Trpzip-2c obtained with different *T*-jump magnitudes, as indicated, from different initial temperatures to the same final temperature (61.8 $^{o}$C).

**References**

1) Xu, W.; Lai, Z.; Oliveira, R. J.; Leite, V. B. P.; Wang, J. *J. Phys. Chem. B* **2012**, *116*, 5152.
2) Xu, Y.; Purkayastha, P.; Gai, F. *J. Am. Chem. Soc.* **2006**, *128*, 15836.